PRODUCTION OF NITRATE FROM ROOTS AND ROOT NODULES OF LUCERNE AND SUBTERRANEAN CLOVER.

By H. L. Jensen, Macleay Bacteriologist to the Society,
and Dorothy Frith, B.Sc.Agr.
(*From the Department of Bacteriology, University of Sydney.*)

(One Text-figure.)

[Read 25th October, 1944.]

Introduction.

Trumble and Shapter (1937) showed that when grass (*Phalaris tuberosa*) was grown in almost nitrogen-free sand together with annual legumes (*Medicago denticulata* or *Trifolium subterraneum*) there was no evidence of any transfer of fixed nitrogen from the legume to the grass during active growth of both plants, but that the growth of the grass was rapidly stimulated after removal of the tops of the legumes. The authors ascribed this phenomenon to release of available nitrogen from the decaying root systems of the legumes; their data, however, show that only a moderate proportion (30 per cent., or less) of the total nitrogen contained in the legume root systems had been taken up by the grass. An important factor governing the utilization of this nitrogen is, obviously, the rate at which the organic constituents of the roots and nodules are decomposed and their nitrogen converted into ammonia and nitrate; at the same time the possibility exists that soluble organic nitrogenous compounds may be utilized by the grass before they reach the inorganic stage (Nicol, 1934); such compounds might represent either native constituents of the legume tissues, or products of the microbial decomposition processes. Since the root nodules differ considerably from the rest of the root tissues in chemical composition (Fred *et al.*, 1932), it is likely that they also differ in the availability of their nitrogen. Experiments on legumes as green manure to non-leguminous plants are almost innumerable, and several authors, e.g., Whiting (1926), Whiting and Richmond (1927), and others quoted by Fred *et al.* (1932), have studied the comparative rates of nitrification of tops and roots of different legumes, but in none of these contributions is any distinction made between the nodules and the actual roots. We have therefore carried out some such experiments with materials representing two important pasture legumes, viz., lucerne (*Medicago sativa*) and subterranean clover (*Trifolium subterraneum*), at an age comparable to the plants in the experiments of Trumble and Shapter (1937).

Experimental.

Methods.—The plants that yielded the materials were grown for 3 to 4 months under greenhouse conditions in sand very poor in nitrogen, some of them also in soil of fairly low humus content, and all inoculated with effective strains of the corresponding root-nodule bacteria. Materials from different experimental series had to be combined in order to get a sufficient supply of nodule substance. Table 1 shows the contents of carbon and total and water-soluble nitrogen in the plant substances as used for the subsequent experiments (finely ground, air-dry material). Carbon was determined by elementary analysis, and nitrogen by the Kjeldahl method, with selenium as a catalyst. Water-soluble nitrogen was determined by shaking 1 gm. of material, or 0·2 gm. in case of nodule substance, with 100 ml. of distilled water for 3 hours in a mechanical shaker, filtering, washing with distilled water until 200 ml. of filtrate had been collected, and analyzing the total filtrate for nitrogen. Ammonia and nitrate were determined by the method of Richardson (1938).

TABLE 1.
Composition of Plant Materials.

Material.	In Air-dry Matter, %.		C : N Ratio.	Water-soluble N, %.	
	Total C.	Total N.		In Air-dry Matter.	Of Total N.
Lucerne tops	39·4	2·48	16·0	0·712	28·7
,, roots	40·7	1·58	25·8	0·630	39·8
,, nodules ..	40·5	5·94	6·8	—[1]	—
Clover tops	40·7	2·63	15·5	0·270	10·2
,, roots	37·6	2·20	17·1	0·364	16·7
,, nodules	47·3	7·42	6·4	2·210	29·8

[1] Insufficient material available for the determination.

Rate of Nitrification.—The nitrifiability of nitrogen in all six materials was tested in a mixture of sand and loam soil, of pH 7·2 and very low nitrogen content (0·019% Total-N), to which the materials were added in quantities equivalent to 50 p.p.m. of nitrogen. Duplicate portions of soil were adjusted to approximately 12% moisture content and incubated at 30°C. in glass jars covered with Petri dishes; losses of moisture were restored every two or three days by addition of distilled water, and ammonia and nitrate were determined after 2, 5 and 8 weeks. No measurable quantities of ammonia were found; therefore only the figures for nitrate are given in Table 2; the percentage nitrification of nitrogen in the added plant substances is calculated as excess over the average of the two control jars.

TABLE 2.
Production of Nitrate from Leguminous Plant Materials.

	NO_3-N, p.p.m., after			Percentage of added N Nitrified after	
	14 Days.	35 Days.	56 Days.	14 Days.	35 Days.
Lucerne tops. (*a*)	6·6	28·8	28·4	10·4	53·0
(*b*)	7·6	27·0	29·1	12·4	49·4
,, roots. (*a*)	2·2	19·1	20·5	1·6	33·6
(*b*)	1·3	17·9	19·4	(0)	31·2
,, nodules. (*a*)	14·9	46·2	30·4	27·0	87·8
(*b*)	15·1	46·7	34·4	27·4	88·8
Clover tops. (*a*)	9·4	26·5	33·8	16·0	48·4
(*b*)	7·7	25·9	29·6	12·6	47·2
,, roots. (*a*)	8·5	21·0	33·2	14·2	37·4
(*b*)	7·7	24·9	29·4	12·6	45·2
,, nodules. (*a*)	12·7	47·2	23·4	22·6	89·8
(*b*)	12·7	45·4	33·0	22·6	86·2
Control. (*a*)	1·8	2·7	16·9	—	—
(*b*)	1·0	1·9	14·4	—	—

After 2 and 5 weeks, the two nodule materials show the most rapid nitrification, which reaches almost 90%, and the lucerne roots the slowest, while the tops and the clover roots occupy intermediate positions. After 8 weeks, the results become inconclusive, because a marked loss of nitrate has taken place in the jars with nodule substance, and the control jars show a sudden increase in nitrate which makes it difficult to gauge the nitrification of the other materials; it is by no means certain that an equally strong production of nitrate from the soil's own organic matter would have taken place in the presence of added organic matter. Yet the rather small increase in nitrate content between the fifth and eighth week suggests that the nitrification of the residual nitrogen in tops and roots now takes place at only a slow rate.

After 2 and 5 weeks, there is a close negative correlation between nitrification and C:N ratio of the materials. By calculating the correlation coefficients between C:N ratio and percentage nitrification for the two sets of duplicate jars, and combining them into one by calculating the corresponding values of z (Fisher, 1936), we find:

	Correlation Coefficient (r).	n	P
After 2 weeks	−0·810	6	0·02—0·01 (significant)
„ 5 „	−0·789	6	0·02—0·01 „

Several authors, e.g., Whiting and Richmond (1927) have reported a positive correlation between the rate of nitrification and the amount of water-soluble nitrogen in the materials. Our data give no indication of this; indeed, the nitrogen in lucerne roots is nitrified most slowly of all, in spite of the fact that this material contains more soluble nitrogen than any other, and the nitrogen in clover tops is nitrified as rapidly as that in lucerne tops which are nearly three times as rich in soluble nitrogen.

Vegetation Experiments.—Direct utilization of nitrogen in some of the materials (clover nodules, and clover and lucerne roots) by wheat plants was tested in a supplementary experiment. Quantities equivalent to 40 p.p.m. of nitrogen were added to duplicate 600-gm. portions of sand mixed with 20% of the same soil as used in the nitrification experiments, and 0·3 gm. $CaHPO_4$, 0·06 gm. KCl and 0·06 gm. $MgSO_4$ were added as mineral fertilizers. The sand medium was placed in the same jars as previously used, 15% of water was added, and four grains of wheat ("Warigo") were sown per jar; after germination the seedlings were thinned to two per jar. The plants were grown for eight weeks (17th April to 13th June, 1944) under greenhouse conditions, and were then analysed for nitrogen *in toto* (tops and roots carefully washed free from sand). After removal of the plants the sand-medium was air-dried, readjusted to 10% moisture, returned to the jars, and incubated for 4 weeks at 30°C. in order to test the availability of the nitrogen that had not been taken up by the plants. No ammonia, and only traces of nitrate, were shown by qualitative tests immediately after harvesting of the plants. The contents of nitrogen in the wheat plants and the subsequent formation of nitrate is shown in Table 3; as in the previous nitrification experiment, no accumulation of ammonia took place.

TABLE 3.

Assimilation by Wheat Plants, and Subsequent Nitrification, of Nitrogen in Roots and Root Nodules.

Source of Nitrogen.	N in Plants, Mgm.		NO_3-N Produced, P.p.m.		Percentage of added N made Available.		
	Total.	Excess over Control.	Total.	Excess over Control.	Assimilated by Plants.	Nitrified.	Total.
Clover nodules. (*a*)	13·3	9·2	7·3	3·4	38·3	8·3	46·8
(*b*)	13·6	9·5	6·8	2·9	39·6	7·3	46·9
„ roots. (*a*) ..	8·0	3·9	4·3	0·4	16·4	1·0	17·4
(*b*) ..	7·8	3·7	4·1	(0·2)	15·4	(0·5)	15·9
Lucerne roots. (*a*)	5·6	1·5	5·5	1·6	6·3	4·0	10·3
(*b*)	5·8	1·7	4·8	0·9	7·1	2·3	9·4
Control. (*a*) ..	3·9	4·1	3·7	3·9			
(*b*) ..	4·3		4·1				

The analysis of the wheat plants, as well as their appearance at the time of harvesting (Fig. 1), shows a definite superiority of the nodule-nitrogen, of which nearly 40% has been utilized, while clover-root nitrogen is less than half as effective, and the availability of lucerne-root nitrogen is barely measurable; this agrees with the results of Parbery and Swaby (1942), who found that only such materials which contained at least 2·0 to 2·5% nitrogen in dry matter gave any benefit to rye grass in pot experiments. Very little available nitrogen was provided by the medium itself, as shown by the fact that

Fig. 1.—Wheat plants with different sources of nitrogen. From left to right: Control (no N); 24 mgm. N as lucerne roots; do. as clover roots; do. as clover nodules. Age of plants: 8 weeks.

the control plants contained only two to three times as much nitrogen as originally present in the seed which contained 1·52 mgm. N per two grains. Also in the subsequent nitrification test, the residual nitrogen of the nodules undergoes some nitrification, while that in the two root materials is only very slowly transformed; especially the clover-root substance seems to have yielded all its readily available nitrogen to the wheat plants.

Discussion.

The results show clearly that the nitrogen in root nodules is much more rapidly transformed into plant food than that in the rest of the root system. As seen by comparison with data given by Fred *et al.* (1932) and Wilson and Westgate (1943), the nodule substances used in the present experiments show a high, although not exceptional, percentage of nitrogen, so that presumably their C:N ratios would be narrower, and the nitrifiability higher, than would be the case with most nodule materials. But since nodule material is mostly, and probably always, richer in nitrogen than the corresponding root material, we may expect the general rule to apply that dead root nodules represent a source of nitrogen which readily becomes available to non-leguminous plants, while the root substance proper yields up its nitrogen more gradually, probably owing to its relatively higher content of organic carbon which serves as energy material for micro-organisms temporarily immobilizing some of the nitrogen. In the experiments of Trumble and Shapter (1937), the nitrogen responsible for the improved growth of grass after cutting the tops of associated legumes, thus probably originated from the root nodules rather than from the roots themselves. On the other hand, the long-term effect of legumes in increasing the yield of subsequent cereal crops (Nicol, 1933) may be ascribed largely to the more slowly mobilizable nitrogen of the root substance proper. The superiority of lucerne in this respect, as observed by Nicol, may be due to the fact that lucerne contains a larger proportion of root-nitrogen than the annual legumes. This, as well as the fact that a considerable proportion of the plants' total nitrogen content is represented by the nodule substance, may be seen from the following data which show the distribution of nitrogen in the plants from which the materials used in our experiments were derived, besides a few others also grown for 3 to 4 months in sand or in soil of low humus content:

	Percentage of Plants' Total Nitrogen in		
	Tops.	Roots.	Nodules.
Medicago sativa	50–66	29–43	4·5–12·5
Medicago tribuloides	80–82	13–15	3·4– 7·2
Trifolium subterraneum	69–85	11–21	3·6–12·6
Trifolium repens	81–84	11–14	3·5– 4·4

Obviously even a partial release of the nitrogen in the nodules might result in a perceptible stimulation of associated non-legumes. Such a release might take place not only from entire root systems left behind by dead leguminous plants, but also from nodules lost during active growth. As shown by Wilson (1931, 1942), clipping of the tops as well as intermittent drought causes shedding of a certain proportion of the nodules, the nitrogen of which will thus be rendered available. This phenomenon may not only be of importance under field conditions, but may also exist in pot experiments, and represents one of the many factors to be taken into consideration in experimental work on the excretion of nitrogenous compounds from the root systems of leguminous plants.

Summary.

The nitrogen in root nodules of lucerne and subterranean clover was nitrified more rapidly than that in the tops, and this again more rapidly than the nitrogen in the root tissues proper. Nearly 90 per cent. of the nodule-nitrogen could be converted into nitrate within 5 weeks at 30°C. The rate of nitrification showed a significant negative correlation with the C:N ratio of the substances, but showed no correlation with the content of water-soluble nitrogen. Experiments on the availability of root- and nodule-nitrogen to young wheat plants gave results in agreement with the nitrification tests. The nitrogen of dead root nodules thus seems readily to become available to non-leguminous plants, while the root substance itself represents a more slowly mobilizable reserve of nitrogen.

Acknowledgements.

We wish to thank Mrs. Daphne M. Buckley, B.Sc., Department of Organic Chemistry, for the carbon determinations, and Mr. S. Woodward-Smith, Department of Medical Artistry, for the photograph.

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